AD_____

Award Number: W81XWH-11-2-0017

TITLE: Monitoring Tissue Ischemia After Potentially Life Threatening Post-Traumatic Injuries

PRINCIPAL INVESTIGATOR: Dr. Wanchun Tang

CONTRACTING ORGANIZATION: Institute of Critical Care Medicine
Rancho Mirage, CA 92270

REPORT DATE: December 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-12-2011	Annual	23 Nov 2010 - 22 Nov 2011

4. TITLE AND SUBTITLE

Monitoring Tissue Ischemia After Potentially Life Threatening Post-Traumatic Injuries

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-2-0017

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Dr. Wanchun Tang

E-Mail: drsheart@aol.com

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Institute of Critical Care Medicine
Rancho Mirage, CA 92270

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Hemorrhagic shock remains the dominant cause of death after both battlefield and civilian trauma. The conventional management of fluid resuscitation titrated to normal levels of blood pressure may increase mortality. Fluid resuscitation during hemorrhagic shock is therefore best based on more specific measurements indicating adequacy or inadequacy of tissue perfusion rather than on arterial (blood) pressure levels. This research was to develop and optimize a nanosensor as a monitoring device of tissue PCO2 and to examine the measurement of sublingual PCO2 (PSLCO2) as a quantitative indicator of tolerable or intolerable acute blood loss as a basis for refining therapeutic strategies for volume repletion. The first stage of the study was to develop a PSLCO2 sensor based on the existing Sensation platform. The Sensation carbon nanotube sensor technology provided a flexible platform with improved signal to noise ratio and accuracy utilizing a simple reader design with low power requirements. In second stage, two phases of studies will be performed on a porcine model of hemorrhagic shock. The measurement of PSLCO2 provided a significantly better indicator of the severity of tissue ischemia and there better guide for optimizing fluid resuscitation. It is especially significant for the military application since it will avoid unnecessary fluid replacement when the resource is extremely limited in the battlefield.

15. SUBJECT TERMS

hemorrhagic shock, tissue perfusion, tissue PCO2, resuscitation

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	11	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Introduction

Hemorrhagic shock remains the dominant cause of death after both battlefield and civilian trauma. The conventional management of hemorrhagic shock is early and aggressive volume repletion such as to restore normal blood pressure as the predominant measurement. However, fluid resuscitation titrated to normal levels of blood pressure may increase severity of tissue ischemia and thereby increase mortality. Fluid resuscitation during hemorrhagic shock is therefore best based on more specific measurements indicating adequacy or inadequacy of tissue perfusion rather than on arterial (blood) pressure levels. No disposable low cost, rapid response, long shelf life, continuous measuring method is currently available. This application is to develop and optimize a nanosensor as a monitoring device of tissue PCO_2 and to examine the measurement of sublingual PCO_2 ($P_{SL}CO_2$) as a quantitative indicator of tolerable or intolerable acute blood loss as a basis for refining therapeutic strategies for volume repletion. We hypothesize that (1) a CO_2 nanotube sensor could be developed for the measurement of $P_{SL}CO_2$ with the advantages of small in size, minimal drift, rapid response, and provide real time and accurate measurement of $P_{SL}CO_2$, and (2) resuscitation guided by the threshold levels of $P_{SL}CO_2$ improves outcomes in comparison with resuscitation guided by blood pressure.

Body

The following studies were performed and completed:

Phase I. Detailed Planning: Several kickoff meetings between WICCM and Nanōmix team members were conducted to review amend and finalize the specifications of the sensor. Resource planning and acquisition was taken place during this phase. Sensors, reagents and the project specific analytical equipment were sourced, designed or constructed as part of the transition to Phase II.

Phase II: Electrochemical Investigation:

The aims of the phase II studies were: 1) Obtain and compare different CO_2 enzymatic detection systems. 2) Establish basic electrochemical parameters on the FET (field effect transistor platform). 3) Basic assay parameters to establish a local optimum for assay conditions. 4) Performance evaluation to finalize interim detection candidates for taking to Phase III. 5) Output: Identification of at least one catalytic system that can meet the performance requirements for $P_{SL}CO_2$ measurement.

The following studies were completed: 1) Obtain and compare different CO_2 enzymatic detection systems: Carbonic anhydrases (CAs) catalyze the reversible reaction of $CO_2 + H_2O = HCO_3^- + H^+$, which is fundamental to many processes such as respiration, renal tubular acidification and bone resorption. We evaluated 5 enzymes (human CA1, 4, 5A, bovine and mouse CA) that span functional (e.g. location of secretion) and taxonomic (across species) spectra. Human enzymes were selected. Initially enzymes were attached to the sensors using direct adsorption. 2) Establish basic electrochemical parameters on the FET (field effect transistor platform): The gate voltage sweep was conducted using a triangular wave-form with three parameters: amplitude, frequency and offset. A wide range of these parameters with fewer parametric combinations were explored initially in conjunction with the enzyme survey. The optimal combination of platform and enzymes was selected. 3) Basic assay parameters to establish a local optimum for assay conditions: Using the results of the initial functional survey, we designed a finer scaled Design of Experiment model for local optimization. 4) Performance evaluation to finalize interim detection candidates for taking to Phase III: The optimal combination of platform and enzyme were used in bench protocols designed to determine initial estimates of precision,

reproducibility, stability and response time. 5) Output: A catalytic system that can meet the performance requirements for $P_{SL}CO_2$ measurement was identified.

Phase III: Sensor Platform Investigation:

The aims of the phase III studies were: 1) Compare assay conditions identified in Phase II using multiple enzyme functionalization schemes. 2) Performance evaluation to finalize detection candidates for taking to Phase IV 3) Output: standardized sensor functionalization protocol.

The following studies were performed and completed: 1) Compare assay conditions identified in Phase II using multiple enzyme functionalization schemes: The enzyme candidates listed in Phase II were used in a series of experiments designed to evaluate the impact of functionalization methodology. There are currently two basic ways to immobilize the enzyme to the CNT sensor, direct adsorption and covalent attachment. Either of these can be conducted using intermediate linkers as well. Selection of the final method was based upon performance. The ability of the enzyme to survive the functionalization process and remain attached to the CNTs was the critical outcome of this analysis. Based on the results of the studies, the adsorption technique was selected (Fig. 1). We also considered new sensor designs conceived during the Phase I and Phase II. A flexible substrate platform was selected (Fig. 2). The advantage of this system was the lower cost and shorter turnaround time on design iterations. We performed initial experiments to determine the feasibility of this platform to detect changes in local pH. 2) Performance evaluation to finalize detection candidates for taking to Phase IV. As above, the top candidates were used in protocols designed to determine initial estimates of precision, reproducibility, stability and response time. 3) Output: standardized sensor functionalization protocol was developed.

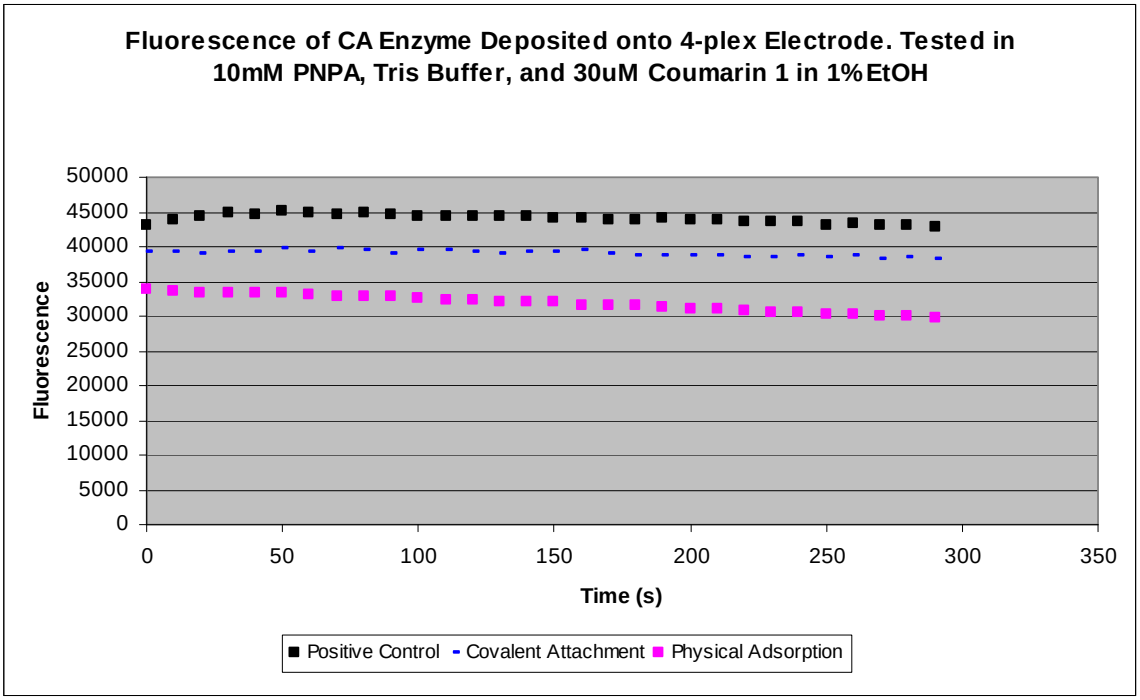

Fig. 1

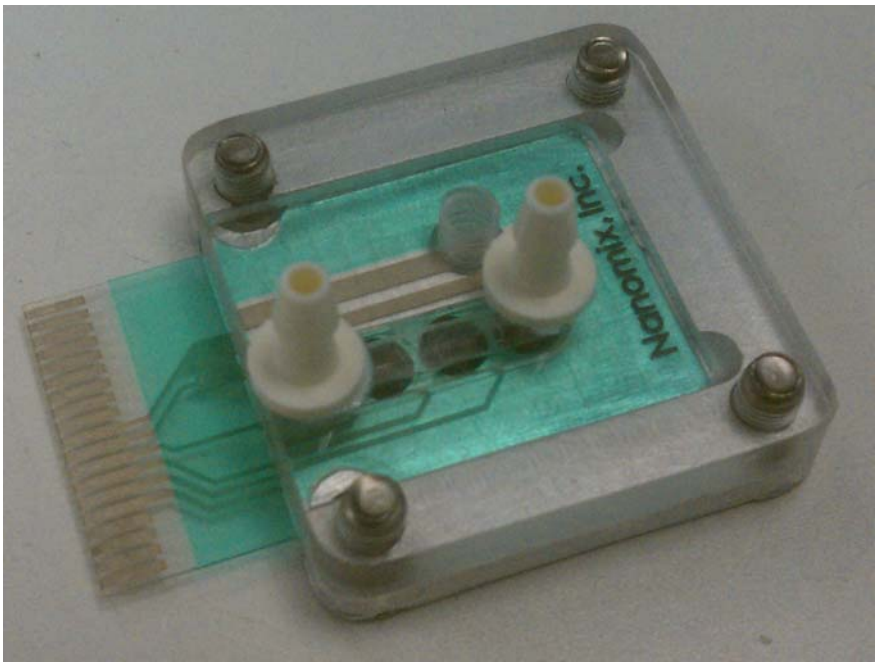

Fig. 2

Phase IV: Systems Integration:

The aims of the phase IV study were: 1) Evaluate alternative sensor localization methods. This could include CO_2 selective membranes vs. completely open system. 2) Evaluate power and data processing designs. 3) Performance evaluation of best performing integrated systems for taking to Phase V. As above, the top candidates will be used in protocols designed to develop initial estimates of precision, reproducibility, stability and response time.

The following studies were performed and completed: 1) Evaluate alternative sensor localization methods. This could include CO_2 selective membranes vs. completely open system. Our previous experience with the FET sensor had focused on detection in the gas phase. For tissue measurement in liquid we need the addition of a gas permeable membrane. For this study, a modified sensor and membrane fixture were used (Fig 3 and 4). The silicon membrane was initially selected. The response time to CO2 was no different with or without membrane (Fig 5). However, the recovery time was unacceptably long (Fig 6). Further reduced the thickness of the silicon membrane resolved this issue. The next technical challenge was the baseline drift. After the sensors expose to increments of CO2 concentrations (4, 6, and 8%), the reading often failed to return to baseline (Fig 7). The initial hypothesis was that this may be caused by the membrane leak. However, extensive testing with dry assembly and 12h emersion leak test excluded that leak was the cause of the drift. We then learned that this drift is caused by the contamination of the iron particles from the mixing step during manufacture. Since there is currently no iron particle free silicon membrane available, we decided to use Teflon as the membrane. The initial results were encourage and the drift issue is now minimized (Fig 8). 2). A prototype box for signal conditioning was produced (Fig 9). The software for data processing was also designed which digitized the measurements with precision, reproducibility, stability and good response time.

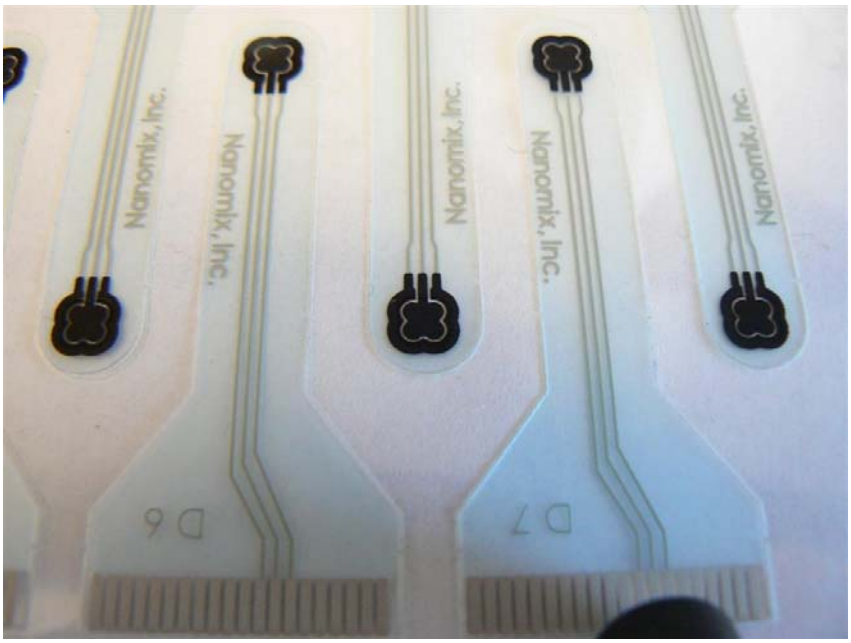

Fig. 3

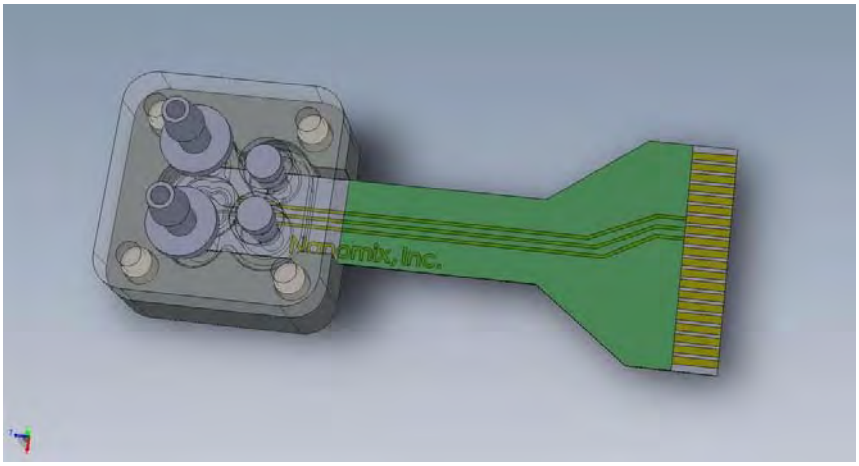

Fig. 4

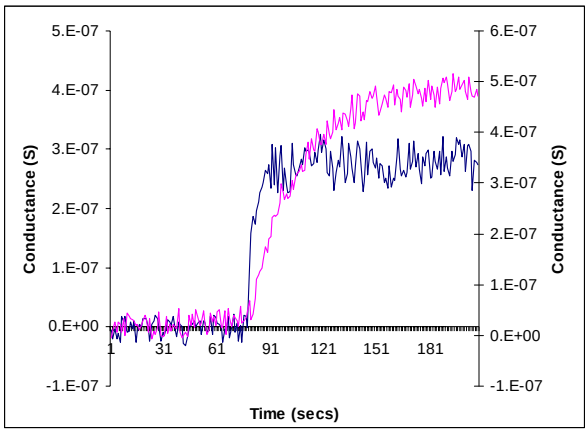

Fig. 5

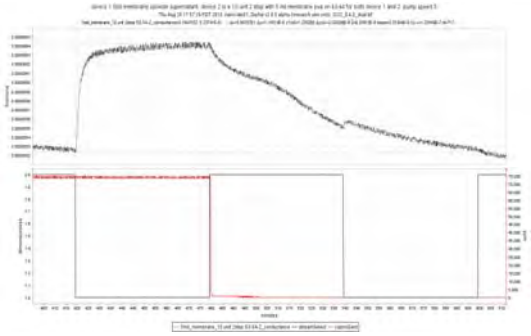

Fig. 6

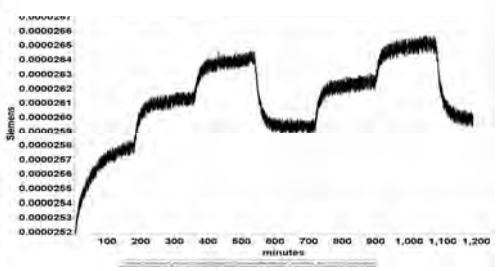

Fig. 7

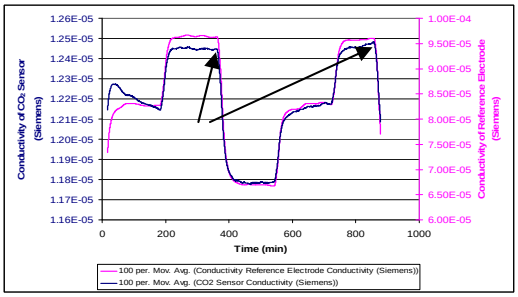

Fig 8

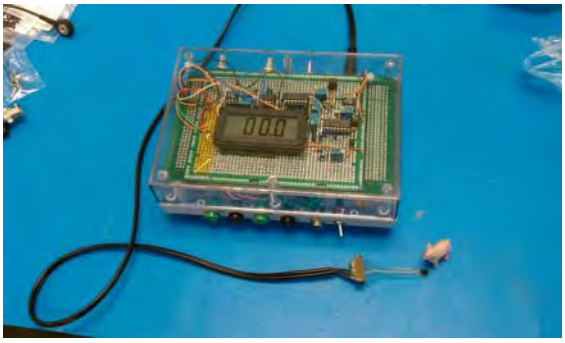

Fig 9

Phase V: Prototype Design, Manufacture and Evaluation:

The aims of the phase V study were: 1) Convert bench-top proof of concept designs generated in Phase IV to prototypes with several iterations of design and testing. 2) Finalize prototype designs and build units for animal testing. 3) Performance evaluation of final prototype.

The following studies were performed: 1) Convert bench-top proof of concept designs generated in Phase IV to prototypes. The first task was to miniaturize the conceptual device so that it could fit into the sublingual area of the testing animals and human patients. In the design, the integrated sensor device is to be packaged with electrical connections to a display device. The prototype unit is consisted a functionalized sensor, a sensor package and enclosure, a package compatible socket, a shielded wire, a printed-circuit board, tubing and filter media. Data are read from a serial port on the printed circuit board. The size of the sensors was reduced to fit our specifications. However, the first problem we encountered was a inconsistent response time as shown in the following figure 10:

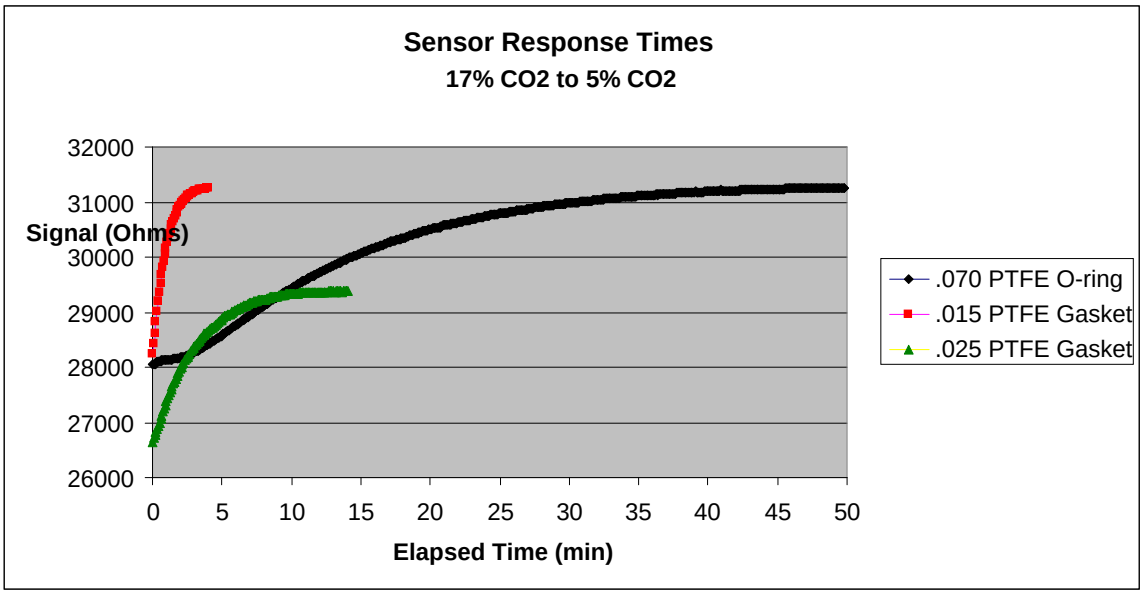

Fig. 10

Sensor ID	Full Transition Time	95% Transition Time (T95)
2A-B7 (**Black**)	49.8 min	34.8 min
3A-B8 (**Red**)	4.0 min	2.5 min
4A-B9 (**Green**)	14.1 min	8.2 min

We then recognized that the current sensors were constructed of hand-made, research components. We therefore optimized design tolerances and sensor materials. We also developed and implemented a predictive algorithm in the software which further reduced the response time as shown in the following figures 11 and 12:

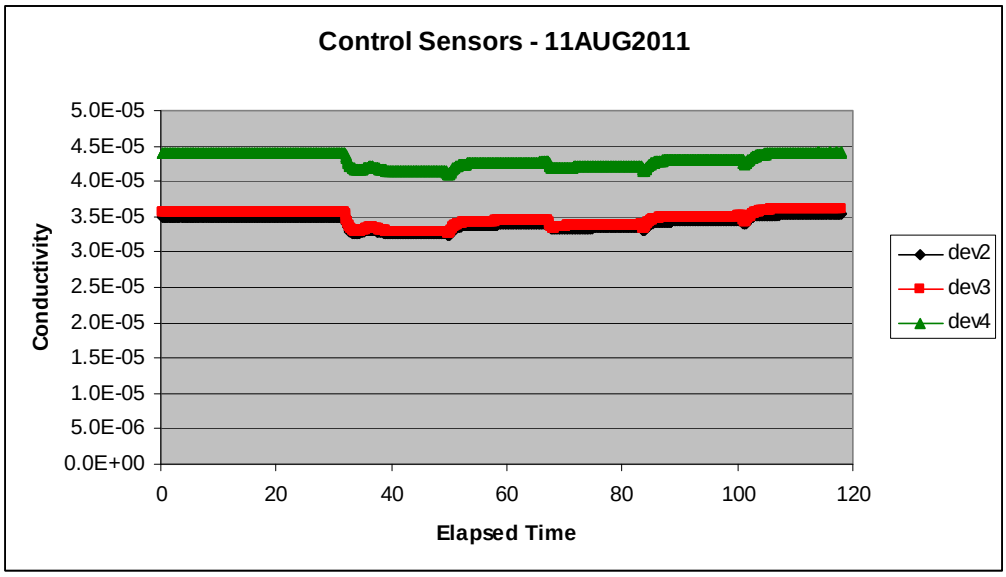

Fig. 11

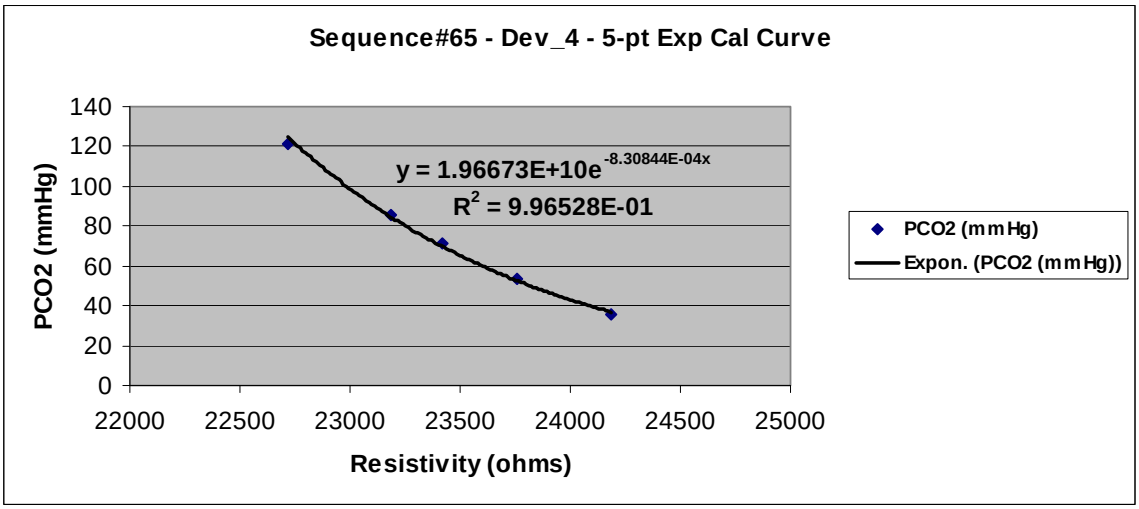

Fig. 12

In the design, the integrated sensor device is to be packaged with electrical connections to a display device. The prototype unit is consisted a functionalized sensor, a sensor package and enclosure, a package compatible socket, a shielded wire, a printed-circuit board, tubing and filter media. Data are read from a serial port on the printed circuit board. The size of the sensors was reduced to fit our specifications. Since CO2 measurement is highly sensitive to the temperature changes. To avoid the potential error due to temperature changes during in vivo studies, a temperature sensor was incorporated into the CO2 sensor. Software was developed to correct the measurements based on temperature changes. We are now able to obtain consistent measurements in a wide range of temperature as shown in the following figure 13:

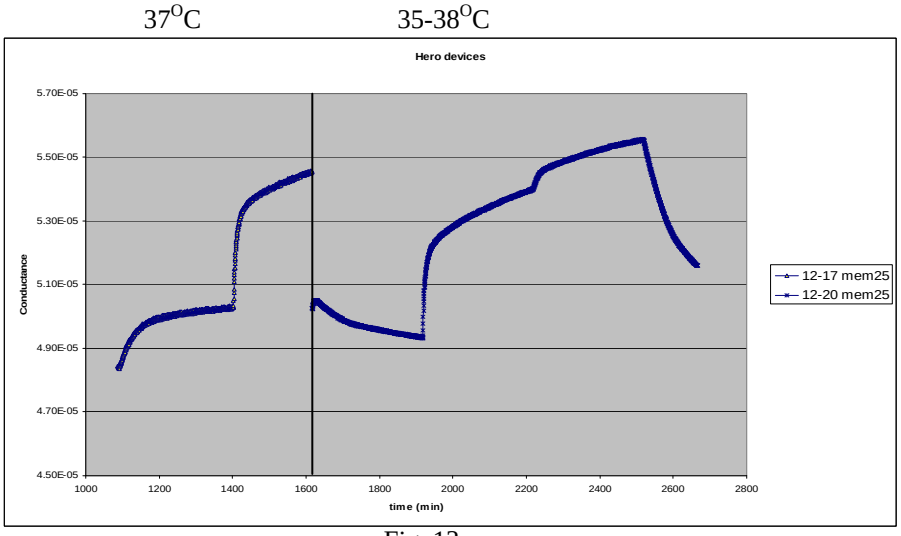

Fig. 13

The unit is going to be interfaced with Weil Institute's computer system during the next 2 weeks. The animal studies has been reapproved by IACUC and will be initiated the beginning of January 2012.

Key Research Accomplishments

We have completed the development of a low cost, pre-calibrated disposable sensor as a monitoring device, which measures the CO_2 that is diffused from mucosal surfaces, such as to quantitate the severity of tissue ischemia associated with shock states and initially hemorrhagic shock. The prototype device is now ready to be tested in animal model of hemorrhagic shock.

Reportable Outcomes

There is currently no publication resulted from this study. We anticipate that several full length manuscripts to be developed after we complete the phase VI studies.

Conclusion

The sensor is now developed and ready to be tested in our pig model of hemorrhagic shock. We do not anticipate any delay for completing the phase VI study.